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The intricacies of dinoflagellate pellicle cysts: The example of *Alexandrium minutum* cysts from a bloom-recurrent area (Bay of Baiona, NW Spain)

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ABSTRACT

The terms "temporary", "pellicle", and "ecdysal" cyst have been employed arbitrarily in the literature of the dinoflagellate life cycle to describe a non-motile and single-layered-wall stage with no mandatory dormancy period, of asexual or sexual origin. These three terms have been used more or less synonymously, but more specific definitions, taking into account morphological and physiological aspects and their roles in dinoflagellate population dynamics, are still needed. To clarify the current terminology, we examine and discuss the usages and foundations of those terms. The background for this discussion is provided by a comparison of the morphology and germination times of three different types of *Alexandrium minutum* cysts collected during a seasonal bloom in the Bay of Baiona (NW Spain). The double-walled cysts were similar to the resting cysts reported for this species, but other, thin-walled and thecate cysts were also observed. These latter cyst types needed between 1 and 17 days to germinate and were therefore considered as short-term cysts, in contrast to the 1.5-month dormancy period of resting (hypnozygotic) cysts. Our results showed that the temporal distribution of these short-term cysts during the bloom period followed a pattern very similar to that of vegetative cells. However, resting cysts were only detected at the end of the bloom. In the context of our present knowledge regarding the dormancy and quiescence of dinoflagellate cysts, "temporary" is a very misleading and uncertain term and must be rejected. The term "ecdysal" has been used in reference to thin-walled cysts when ecdysis has been proven; however, ecdysis is not unique to this type of cysts as thick-walled zygotic cysts can be formed thorough ecdysis of a thecate planozygote. In conclusion, based on our current understanding of cysts, the term "pellicle" more appropriately describes single-layered-wall stages.

1. Introduction

Fossil dinoflagellate cysts have been widely studied by palaeontologists since the 1930s. Those studies generated a large morphological terminology as well as the development of techniques related to the isolation and identification of cysts (Dale, 1983). It was not until the 1960s that the processes involved in dinoflagellate cyst formation began to be clarified, although today many aspects are still unknown. Indeed, very recent studies provided evidence that the life-cycle transitions of dinoflagellates may be more complicated than was previously thought (Figueroa et al., 2008a). Furthermore, there is a need to more precisely define the terminology used to describe the different cyst stages adopted by the same species, because in some cases function and morphology are coupled.

Basically, two types of benthic forms have been described in the life cycle of dinoflagellates: (i) a double-walled cyst, regarded as a dormant resting cyst with a mandatory dormancy period (Anderson and Wall, 1978); (ii) a non-dormant cyst (i.e., without a mandatory dormancy period), with a single-layered wall, referred to in the literature as an ecdysal, pellicle, or temporary cyst (Dale, 1977; Anderson and Wall, 1978; Taylor, 1980). Thus, according to these definitions, wall features and dormancy are the main defining characteristics used in cyst classification.

Dormancy is defined as the period in an organism's life cycle when growth, development, and physical activity are temporarily suspended by active endogenous inhibition. Dormant cysts are unable to germinate, even under optimal environmental conditions (Anderson, 1980). Dormancy features of dinoflagellate cysts vary depending on environmental conditions such as temperature and nutrient levels (Anderson, 1980; Figueroa et al., 2005). In some species, the conditions in which the planozygotes had been formed were shown to affect dormancy (Figueroa et al., 2005). Furthermore, both the dormancy and the germination of

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some dinoflagellate species may involve an endogenous clock, allowing them to alternate over time between dormancy and quiescence (the suspension of development during unfavorable conditions) (Anderson and Keafer, 1987; Anderson, 1998; Matrai et al., 2005).

Until recently, sexuality and dormancy were always associated with resting cysts, which, accordingly, were also called hypnozygotes. In contrast, thin-walled cysts were generally described in culture, in association with the vegetative cycle and short-term or sudden adverse conditions (see Table 1 for references). However, recent findings revealed that some dinoflagellate species produce asexual resting cysts (Kremp and Parrow, 2006) and that both sexual and asexual life-cycle stages can lead to the formation of ecdysal cysts (Figueroa et al., 2006a). The terms "ecdysal", "pellicle" and "temporary", all have been used to define thin-walled cysts based on the criteria of pellicle-layer wall, no dormancy, and ecdysal origin (Dale, 1977; Anderson and Wall, 1978; Taylor, 1980). These terms were regarded by Dale (1983) as synonymous or overlapping. Since then, they have been arbitrarily used, resulting in a confusing cyst terminology.

At least 48 species have been reported to form thin-walled cysts as part of their life cycle, associated with very different conditions, both in culture and in nature (see Table 1). The fact that in many species these thin-walled stages were associated with stressful culture conditions and had never been reported in the field suggested they might be laboratory artifacts or an unusual response to a certain stimulus, one not encountered in nature (see Table 1 for references). However, there is also evidence in the literature that these cysts are a true part of the species' life history and not simply a culture artifact (for example, Kita et al., 1985, 1993; Garcés et al., 1999; Litaker et al., 2002; Figueroa et al., 2006a, 2008b). But even assuming that this is the case, too little is known about the role played by these thin-walled cysts in the natural population dynamics of its respective species.

In *Pfiesteria piscicida* (Litaker et al., 2002), *P. shumwayae* (Parrow and Burkholder, 2003), and *Alexandrium taylori* (Garcés et al., 1998), thin-walled cysts are part of their asexual mode of reproduction. However, later studies of the *A. taylori* life cycle suggested that sexuality leads to both double-walled (termed "resting") cysts and thin-walled (termed "ecdysal") cysts (Figueroa et al., 2006a). The thin-walled cysts (of asexual or sexual origin) of *A. taylori* contribute to the daily exchange between benthic and planktonic phases, which has been reported to be essential to its bloom dynamics (Garcés et al., 2002). In other species, (e.g., *Lingulodinium polyedrum*), the role of thin-walled cysts in the life-cycle strategy is unknown. The formation of pellicle cysts was described in laboratory cultures of *Alexandrium tamarense* (now *A. fundyense*) exposed to different stressful conditions (Anderson and Wall, 1978). This stage was shown to be of short duration and of asexual origin; thus, according to the authors, it was unlikely to play a significant role in initiating toxic blooms. Thin-walled cysts of *Alexandrium minutum* have been rarely reported in nature (Garcés et al., 2004) or in cultures (Laabir and Gentien, 1999; Bolli et al., 2007; Figueroa et al., 2007; Laabir et al., 2007). They were observed following cell transit in the tract of *Crassostrea gigas* (Laabir and Gentien, 1999; Laabir et al., 2007). The authors demonstrated the survival of vegetative cells and pellicle cysts of this dinoflagellate in mussel and oyster feces and suggested that these processes serve as a potential seed source for subsequent dinoflagellate blooms. Moreover, immobile vegetative cells of *A. minutum* that had formed under unfavorable culture conditions (mainly phosphate depletion) were described as asexual ecdysal cysts by Figueroa et al. (2007).

In light of these observations, the objective of the present work was to contribute to the clarification of the terminology used to describe thin-walled non-dormant cysts, distinguishing between the terms "temporary," "pellicle", and "ecdysal" in order to better define the different processes involved in the formation of these cysts. The background for this discussion is provided by data of *A. minutum* cysts, specially, regarding the germination age, morphology, and abundance features showed by the different cyst types of this species during a seasonal bloom in Baiona Bay, where such blooms are recurrent (Bravo et al., this isssue).

2. Methods

2.1. Study area and sampling

The Bay of Baiona is located in the south of Ría of Vigo, one of the four Rías Baixas situated along the Galician coast, near the northern limit of the NW Africa upwelling system (Fig. 1). Upwelling and downwelling events characterize the hydrodynamics in this area and are consequences of the interactions between along-shore winds and the coastal topography (Fraga, 1981). Upwelling indices were calculated using wind speed and directional data, measured every 6 h at a station located 43°N 11°W in the East Atlantic Ocean (Lavin et al., 1991). The upwelling index estimates the Ekman offshore transport of surface water along a transect parallel to the shoreline. More information about the characteristics of the study area is given in Bravo et al. (2010).

The cyst study was carried out by means of a sediment trap placed at station P in the southern part of Baiona Bay (Fig. 1) from January to December 2007. The trap consisted of two cylindrical plastic collectors (height 30 cm, diameter 5.5 cm, aspect ratio 5.45), one transparent and another opaque, placed in a metal disk that was tied to a surface buoy by a rope such that the trap was always moored at a depth of 1 m below the surface. The buoy was anchored to a floating dock of the Baiona Marina. Settled material from the trap was collected every week, coinciding with phytoplankton sampling at the same station. Cysts were counted either within 2 h of their preparation or kept at 4 °C in the dark and observed within 48 h. Samples from the trap were sieved to retain the 10- to 100-μm size fractions and collected in filtered sea water in a final volume of 50 mL. The cysts were quantified by diluting a 5-mL sample three times with filtered seawater, with a 1-mL subsample observed alive by inverted light microscopy. Thirty five cysts were isolated; in those in which germination succeeded, the resulting vegetative cells were identified to ensure that the cysts had been identified correctly. Phytoplankton samples were collected at the surface and at 2 m depth using a manual suction pump and fixed with 1% formaldehyde. *A. minutum* was identified by thecal plate tabulation (Balech, 1995) after a few drops of Calcofluor White M2R fluorescent dye (Fritz and Triemer, 1985) had been added to the chamber to stain the plates. The chambers were examined and *A. minutum* counted under 400× magnification using an inverted epifluorescence microscope with UV excitation and with UV filters in place. Temperature and salinity profiles were obtained by a handheld probe system (YSI Model 30M).

3. Results

3.1. Cyst morphology

Cysts of *A. minutum* settled into the traps placed in Baiona Bay during the bloom season of this species. Three types of cysts of *A. minutum* were detected in the traps: (i) double-walled cysts, considered to be resting cysts (Fig. 2A); (ii) thin-walled cysts, regarded as pellicle cysts (Fig. 2B); and (iii) thecate cysts (Fig. 2C). The last two cyst types were determined to be non-dormant cysts

Table 1
Dinoflagellate species for which the formation of pellicle cysts have been reported.

Genus	Species	Field/ Culture	Surrounding conditions	Reference
Alexandrium	*catenella*	F/C	Parasite attack	Delgado (1999)
			Deficiencies in phosphorous	Figueroa et al. (2005)
	hiranoi	F	Life cycle	Kita et al. (1985, 1993)
	minutum	F/C	High cell densities	Garcés et al. (2004)
			Turbulence	Bolli et al. (2007)
			Passage through the digestive tract of oyster	Laabir and Gentien (1999), Laabir et al. (2007)
	ostenfeldii	C	Ageing of cultures	Jensen and Moestrup (1997), Østergaard and Moestrup (1997)
			Parasite infection	Toth et al. (2004)
	peruvianum	C	Life cycle	Figueroa et al. (2008b)
	pseudogonyaulax	C		Montresor (1995)
	tamarense	C	Deficiencies in specific nutrients	Anderson and Wall (1978), Schmitter(1979)
			Changes in temperature	Dale (1977), Fritz et al. (1989)
				Doucette et al. (1989)
				Scarrat et al. (1993)
	taylori	F/C	Life cycle	Garcés et al. (1998, 1999), Giacobbe and Yang (1999)
			Life cycle	Figueroa et al. (2006a)
Amphidinium	*carterae*	C		Sampayo (1985), Kibler (1999)
	klebssi	F	Life cycle	Sampayo (1985)
				Barlow and Triemer (1988)
	operculatum			Murray and Patterson (2002)
	britannicum			Murray and Patterson (2002)
Amyloodinium	*ocellatum*			Brown (1934)
Ceratium	*furca*	F		Okolodkov Y. (personal communication)
	hirundinella	C		Chapman et al. (1982)
Cochlodinium	*polykrikoides*	C	Life cycle	Kim et al. (2002), Kim et al. (2007)
Coolia	*monotis*	C	Induction by bioactive compound	Sakamoto et al. (2000)
Crypthecodinium	*cohnii*		Life cycle	Ucko et al. (1997), Pfiester (1989)
Cystodinium				Pfiester and Lynch (1980)
Disodinium				Elbrächter and Drebes (1978)
Fragilidium	*subglobosum*			Elbrächter and Drebes (1978)
Gambierdiscus	*toxicus*	C	Induction by bioactive compound	Taylor (1979), Sakamoto et al. (2000)
Glenodinium	*foliaceum*	C		Bricheux et al. (1992)
Gymnodinium	*catenatum*	C	Life cycle	Blackburn et al. (1989)
			Bacteria associated with dinoflagellates	Skerratt et al. (2002)
	pseudopalustre		Life cycle	Von Stosch (1973)
Gymnodinium	*pyrenoidosum*			Horiguchi and Chihara (1988)
Gonyaulax		F		Alldredge et al. (1998)
Heterocapsa	*circularisquama*	C	Bacterial attack	Nagasaki et al. (2000)
		C	Contact cells-bialgal cultures	Uchida et al. (1996, 1999), Nagasaki et al. (2000)
		C	Passage through the digestive tract of oyster	Imada et al. (2000)
		C	Virus attack	Tarutani et al. (2001)
	triqueta	F		Olli (2004)
Katodinium	*fungi-forme*			Spero and Moree (1981)
Lingulodinium	*polyedrum*	C	Fluid shear	Juhl and Latz (2002)
		C	Lower temperature and light	Behrmann and Hardeland (1995)
		F		Marasovic (1989)
		C		Hardeland (1994)
				von Dassow and Latz (2002)
				Figueroa and Bravo (2005)
				Mayali et al. (2007)
Paulsenella	*kornamannii*		Life cycle	Drebes and Schnepf (1988)
Peridinium	*inconspicuim*		Environmental stress	Manjares and Fritz (1999)
	quinquecorne			Anderson et al. (1995)
	volzii		Environmental stress	Manjares and Fritz (1999)
Pfiesteria	*piscicida*	C	Life cycle	Litaker et al. (2002), Burkholder and Glasgow (2002)
			Passage through the digestive tract of oyster	Springer et al. (2002)
	shumwayae	C	Life cycle (mitotic division)	Parrow and Burkholder (2003)
Prorocentrum	*lima*	C	Induction by bioactive compound	Sakamoto et al. (2000), Faust (1993)
Prorocentrum	*micans*			Braarud and Rossavik (1951)
Prorocentrum	*minimum*	C	Changes in temperature	Grzebyk and Berland (1996)
Pyrocystis	*sp*			Elbrächter and Drebes (1978)
	noctiluca		It is not know to have a motile stage	Seo and Fritz (2000), Seo and Fritz (2002)
	lunula		Motility in only a brief stage	Seo and Fritz (2002)
Pyrodinium	*bahamense v. compressum*			Buchanan (1968)
Pyrophacus	*steinnii*			Faust (1998)
Scrippsiella	*trochoidea*		Allelopathy *Alexandrium*	Fistarol et al. (2004)
Scrippsiella	*hangoei*	C	Dark conditions	Rintala et al. (2007)
Woloszynkskia	*apiculata*		Life cycle	Von Stosch (1973)

based on their germinating properties. The morphology of the resting cysts from the traps matched the distinctive features of resting cysts originally described by Bolch et al. (1991) – i.e. thick wall and yellow (accumulation) body – and morphologically resembled the resting cysts found in the sediment of Baiona Bay (Bravo et al., 2010), as shown in Fig. 2F. By contrast,

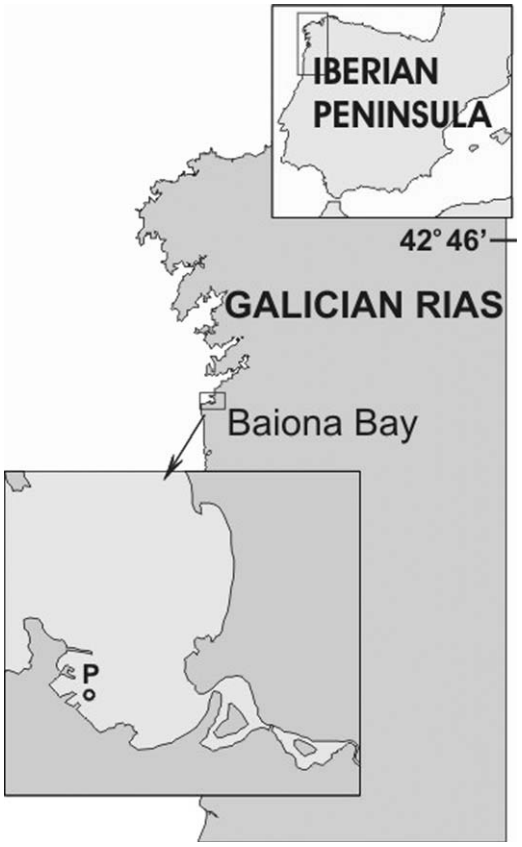

Fig. 1. Map of the Galician coast and the location of the Bay of Baiona, including the station site where this study was conducted.

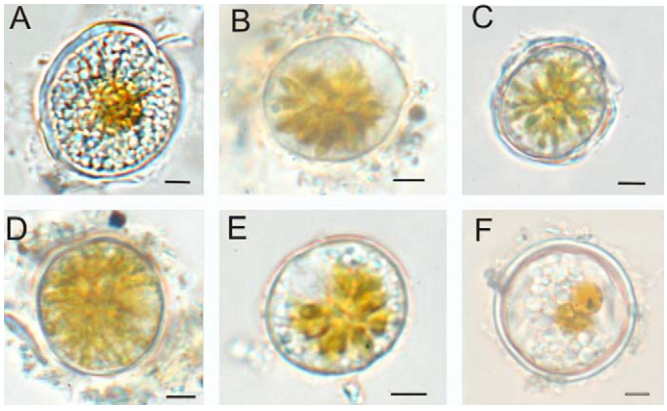

Fig. 2. Resting cysts and pellicle cysts of *Alexandrium minutum*. (A) Resting cyst from the sediment trap, (B) pellicle cysts showing only a thin pellicle layer, (C) pellicle cyst with the theca of the vegetative cell remaining, (D) pellicle cyst with uncondensed chloroplasts, (E) pellicle cyst with condensed chloroplasts, (F) double-walled resting cyst from the sediment. Scale bar: 20 μm.

thin-walled cysts lacked a distinct thick wall and instead were mostly coated by a membrane equivalent to the pellicle layer of the motile stage (Fig. 2B). These characteristics were not entirely consistent for all non-dormant cysts since some were characterized by a wall (Fig. 2D, E), thinner than that of the resting cyst but distinguishable after germination. The germination time of thin-walled cysts was 1–10 days; taking into account that they had an age of 7 days or less, the estimated dormancy period was 1–17 days. In addition, some of the cysts possessed a theca, thus resembling vegetative cells without flagella (Fig. 2C). Isolations of many of these thecate cysts showed that they also germinated within 1–10 days. The appearance of the cytoplasmic content varied from dense and green with numerous chloroplasts (Fig. 2D) to lighter with a more compressed star-shape arrangement of chloroplasts in the center of the cell (Fig. 2E). Finally, the shape of the yellow (accumulation) body differed from that in the resting cysts (Fig. 2A, F), being less condensed. Thin-walled and thecate cysts were also observed attached to seaweed found at Baiona Marina, beside the trap.

3.2. Temporal distribution of cysts and hydrological conditions during the bloom

Pellicle and thecate cysts were present in the traps in a fluctuating pattern, in the same way as vegetative cells. These cysts first appeared in the trap on 19 April, coinciding with the first small bloom of vegetative cells, which eventually reached a concentration of 2.3×10^5 cell L^{-1}. Cyst fluxes increased on 27 April although the vegetative cells had almost disappeared (Fig. 3A). From May until the end of July, vegetative cells reached concentrations of 3×10^3 cells L^{-1}. The concentrations of the vegetative cells and cysts increased in August, but while cyst fluxes were as high as 7×10^3 cysts cm^{-2} day^{-1} on 13 August, vegetative cell concentrations remained below 5×10^4 cells L^{-1}. Another peak in cyst flux took place on September 17, coincident with the last peak of vegetative cells (up to 4.2×10^5 cell L^{-1}) (Fig. 3A). Maximum encystment rates, calculated as the ratio of pellicle and thecate cysts to vegetative cells present in the water during the same period of sampling, ranged between 10^{-2} and 10^{-3}. Resting cysts were also detected in the traps; however, they were only observed during the last peak of vegetative cells, i.e. in September, and persisted for the following 2 weeks, coinciding with cell concentrations of $< 10^4$ cells L^{-1} (Fig. 3B).

The hydrodynamic conditions of Baiona Bay (Fig. 1) are the result of interactions between freshwater input from the Miñor River and offshore influences, dominated by upwelling and downwelling events. Unlike the situation typically found elsewhere along the Galician coast, upwelling episodes were disrupted by downwelling events during the summer of 2007 (Fig. 4A). Freshwater effects were noted mainly during the winter and early spring, until May, with another strong pulse of freshwater in June and smaller influences until the end of August (Fig. 4B). Upwelled water from offshore was characterized by the colder and more saline pulses occurring near the bottom, mainly in August and September (Fig. 4B, C). The first peak of vegetative cells and non-dormant cysts of *A. minutum*, on 19 April, was associated with the change from the winter temperature inversion, caused by the influx of cold freshwater over the warmer seawater, to the summer temperature stratification, occurring for the first time that year (Fig. 4C). This change in the water column came one month after the persistence of positive upwelling indices (Fig. 4A). However, the situation was subsequently interrupted by southwesterly winds, the presence of which coincided with a sudden decrease in the *A. minutum* bloom (Fig. 3A). The temperature and salinity values associated with this maximum in cell concentrations were 13.8–15.3 °C and 33.6–35.3 psu, respectively. During late spring and continuing in summer, only very small concentrations of *A. minutum* were detected until late August, when vegetative cells reached concentrations of up to 5×10^4 cells L^{-1}. In that period, thin-walled cysts appeared in the traps, associated with salinity and temperature stratification in which the water temperature ranged between 14.5 and 18.2 °C. The peak of vegetative cells and thin-walled cysts on 17th September occurred during a period of

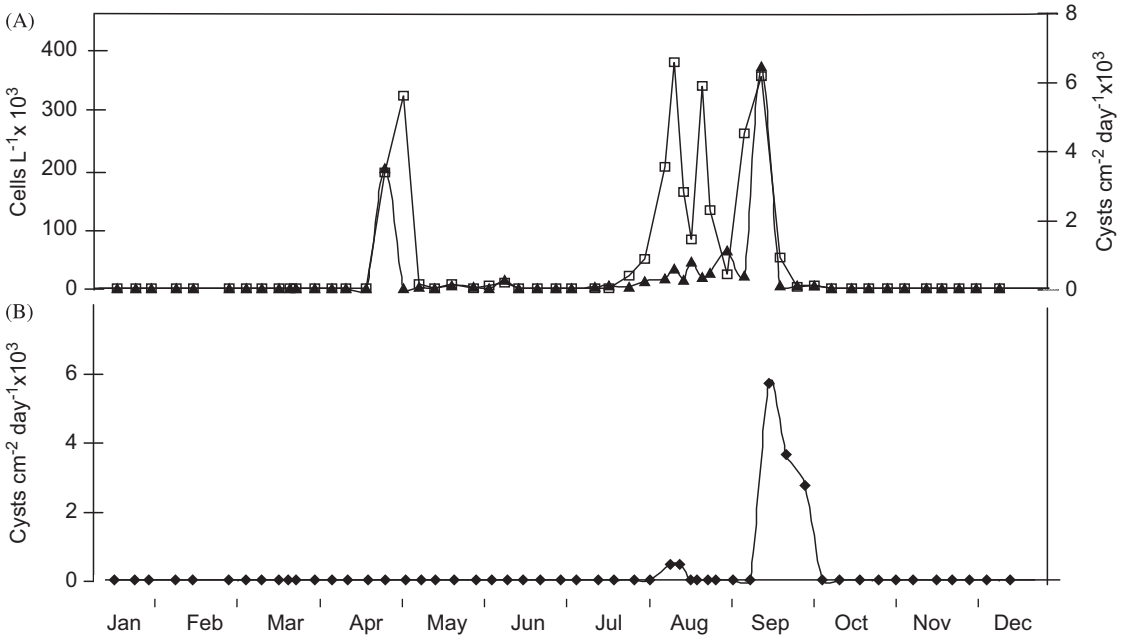

Fig. 3. (A) Vegetative cell concentrations (triangles), pellicle cyst fluxes (squares), and (B) resting cyst fluxes (diamonds) of *Alexandrium minutum* at the Bay of Baiona station.

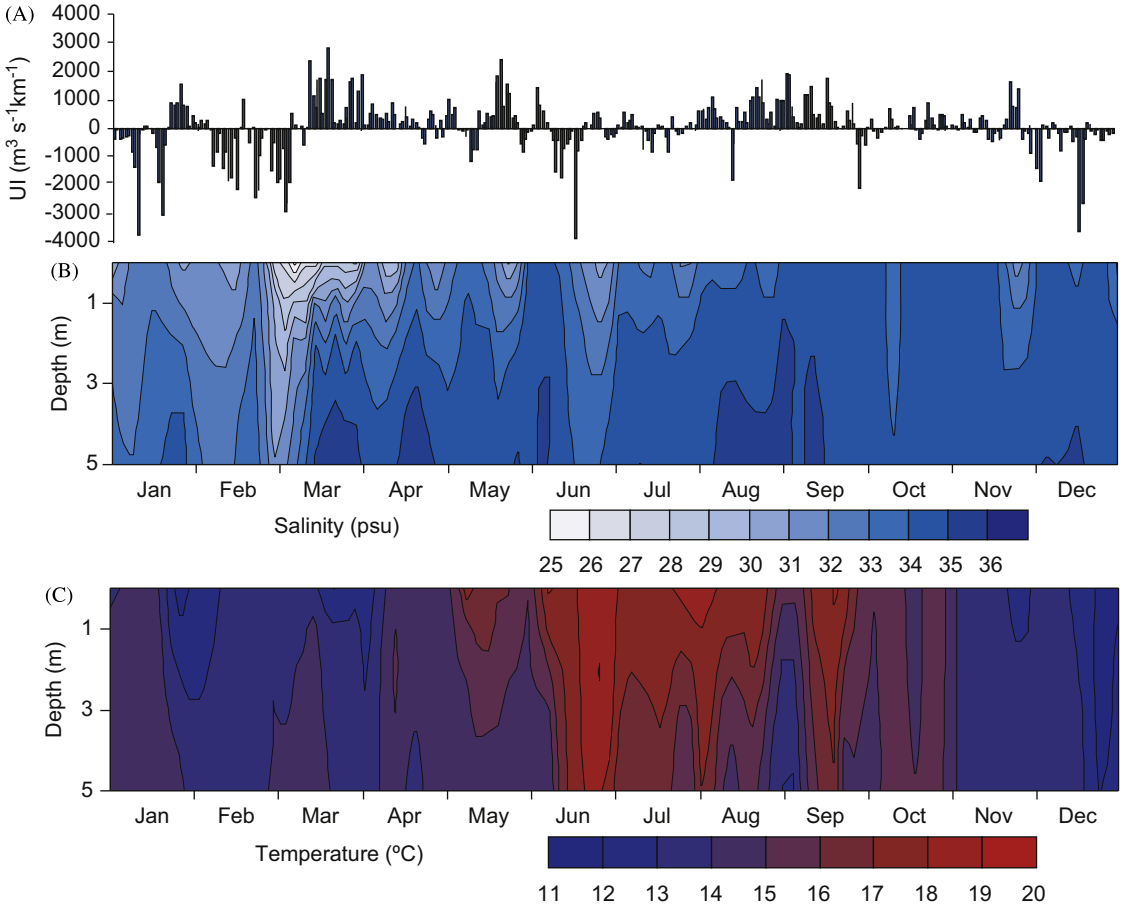

Fig. 4. (A) Upwelling index (UI) and the evolution of (B) salinity and (C) temperature at the Bay of Baiona station. Positive and negative values of UI indicate favorable conditions for upwelling and downwelling, respectively.

moderate positive upwelling indices during which there was thermal stratification of the water column, preceded by several upwelling pulses in August and at the beginning of September (Fig. 4C). The temperature and salinity values associated with this maximum in cell concentration were 17.2–18.2 °C and 34.1–34.8 psu, respectively. As in the spring, the bloom

decreased suddenly, but this time the decrease coincided with the presence of strong northerly winds.

4. Discussion

Remarkable efforts have been recently made to understand and clarify the origins and peculiarities of the different types of dinoflagellate cysts. However, owing to the complexity of the life strategies exhibited by these organisms, the terminology used in the literature to describe thin-walled non-dormant cysts is rather confusing. In our attempts to select the most appropriate term to refer to cysts detected in Baiona Bay, we were confronted with several fundamental questions. For example, are "temporary cyst", "pellicle cyst", and "ecdysal cyst" truly synonymous? "Temporary" and "pellicle" terms were first used in two different studies to describe the same type of cysts formed in *A. tamarense* cultures (at the time called *Gonyaulax tamarensis* or *G. excavata*) (Dale, 1977; Anderson and Wall, 1978). In both studies, these cysts were formed in cultures under conditions of temperature or nutrient stress. Despite their different names, the cysts had the same features: a single-layered wall, non-elongated shape, and the absence of a dormancy period, in contrast to the hypnocysts or hypnozygotes also described by the authors. Pellicle and ecdysal cysts have also been used synonymously (Taylor, 1980; Dale, 1983) but indicate two different aspects of the same process: cells shed the theca, i.e., the ecdysal process, and remain coated by a thin wall equivalent to the pellicle layer of a vegetative cell. Thus, while the three terms have been used to describe the same type of cysts – those that are thin-walled and do not have a mandatory dormancy period – whether this usage is appropriate is discussed below.

"Temporary" makes reference to cysts that do not have a mandatory dormancy period, however it has been frequently used in the literature to describe thin-walled cysts whose germinating time was not determined. Adding to this confusion are the results of studies of the dinoflagellate life cycle. That work, carried out over the last two decades, revealed the complexity of reproductive processes and of cyst dormancy traits (for example, Anderson, 1980; Litaker et al., 2002; Figueroa et al., 2006a). The results of those studies did not contribute to strengthening "temporary" as a suitable term. The main argument is that, as is the case in many organisms, the dormancy period of one dinoflagellate species varies depending on exogenous environmental factors (for example, Anderson, 1980; Rengefors and Anderson, 1998; Figueroa et al., 2005). Thus, despite their critical ecological implications, the factors determining long- or short-term encystment are still unknown for many dinoflagellate species.

The dormancy period of the thin-walled cysts described in the present paper ranged from 1 to 17 days (or 1–10 days if the cysts had formed on the last day of the trap period). Based on this time frame, the *A. minutum* cysts of the present study can be considered as short-term cysts, in contrast to the 1.5-month dormancy period of the double-walled sexual resting cysts from the previous year's bloom in the same area (Figueroa et al., 2007). The ability to quickly recover the mobile vegetative stage should be the important feature of "temporary" cysts, but in our study the cysts did not all behave in the same manner. Furthermore, 10 days is longer than the period reported for the dormancy of sexual double-walled (resting) cysts of other species. For example, the dormancy period of reticulated double-walled cysts of *Gymnodinium catenatum* is only 6 days (Figueroa et al., 2006b) but these cysts were never described as temporary, due to their resistant cell wall. Accordingly, the concepts "temporary" and "resting" should only be considered relative, used in the context of a known, specific life cycle. Indeed, in our opinion, "temporary" is a misleading term and inaccurately describes thin-walled cysts.

"Pellicle" and "ecdysal" might be terms better-suited to describe thin-walled cysts because they do not depend on interpretations of the dormancy period. The first is based on morphological aspect and the second presumes the occurrence of ecdysis during encystment. Moreover, according to our current knowledge, dormancy is not a mandatory feature of these cysts, or at the very least, is significantly shorter than that described for resting cysts of the same species. At this point in the discussion, a few questions arise. Firstly, can a pellicular dinoflagellate cyst be formed by a mechanism different than ecdysis? Ecdysis has been observed in thin-walled-cyst formation during sexual reproduction in *L. polyedrum* and *A. taylori* (Figueroa and Bravo, 2005; Figueroa et al., 2006a) and during the vegetative growth of other species under stress conditions (Anderson and Wall, 1978; Dale, 1983). In thecate dinoflagellates, ecdysis could be presumed to accompany encystment, independent of whether the cysts are formed by sexual or asexual reproduction. However, at least one exception has been reported. Thin-walled cysts of *Pyrodinium bahamense* develop in two ways: (1) by ecdysis from the thecate form and (2) from a gymnodinioid stage that develops during the asexual cycle of this species (Buchanan, 1968). Secondly, do naked dinoflagellates undergo ecdysis when they encyst? Buchanan only reported that the gymnodinioid cells rounded up and acquired hyaline walls, forming what the author called "spherical cysts". This is in contrast to *Gymnodinium pseudopalustre*, whose response to unfavorable conditions includes a loss of motility and the excretion of a well-defined tightly fitting envelope (von Stosch, 1973), probably corresponding to amphiesmal vesicles.

Finally, there is another question that must be considered in distinguishing between the terms "ecdysal" and "pellicle": Is ecdysis restricted to thin-walled-cyst formation or does it also occur during resting-cyst formation in thecate dinoflagellates? Thecate resting-cyst-forming planozygotes have been reported in several species of dinoflagellates, with ecdysis observed in the formation of cysts in several *Alexandrium* species (Figueroa and Bravo, 2005; Figueroa et al., 2005, 2007) but not in that of *Peridinium* species (Pfiester, 1976; Pfiester and Skvarla, 1979). Once again, the diversity of features in the life cycles of dinoflagellates as well as the gaps that still remain in our knowledge are evident. For this reason, we recommend a cautious approach to cyst terminology, one that describes the processes and stages as accurately as possible based on the current knowledge and on the most suitable terminology already reported in the literature. "Ecdysal" refers to a process that does not differentiate between the formation of the two cyst types and hence is not a suitable descriptor for thin-walled cysts. "Pellicle" defines the single-layer-wall cyst stage without other connotations and is, therefore, the more appropriate general term for that kind of cysts. However, there is clearly a need for a more-detailed assessment of the specific processes involved in the great variety of life-cycles of dinoflagellates, including the use of a more specific terminology—one that contributes more information to the definition of a particular process. For example, "division cyst" precisely describes a non-motile vegetative stage of *Woloszynskia apiculata*, *P. piscicida*, *Protoperidinium depressum* and *Protoperidinium steidingerae* that undergoes division (von Stosch, 1973; Litaker et al., 2002; Gribble et al., 2009).

In the present study, we described thin-walled cysts of *A. minutum* collected from traps and compared them with resting cysts acquired from the sediment. In light of our discussion, we refer to them as "pellicle cysts". Non-motile thecate cells that proved to be viable forms were designated as "thecate cysts". Similar forms of this species, observed in association with stress conditions, have been described in the literature. Pellicle cysts

were observed following cell transit in the tract in mussel and oyster feces (Laabir and Gentien, 1999). The authors demonstrated the survival of vegetative cells and postulated that such processes serve as a potential seed source for subsequent dinoflagellate blooms. Immobile thecate vegetative cells of *A. minutum* were described as cysts by Figueroa et al. (2007), in reference to cultures of this species maintained under unfavorable conditions (mainly phosphate depletion). Those authors described germination as the release of the protoplast through the epitheca and giving rise to a naked mobile cell. Such cysts probably correspond to the thecate cysts described in the present paper, based on the observed similarities in their morphological and germination features. Thecate cysts of *A. minutum* were detected in abundance in previous studies of benthic dinoflagellates residing in the seaweed associated with mussel culture rafts in the Vigo ria (data not published). Their isolation and subsequent germination yielded the first cultures of this species in our laboratory.

There exist very few reports in which dinoflagellate pellicle cysts were observed in the field (see Table 1). Instead, these forms are traditionally considered to be non-motile stages, formed by motile vegetative cells in response to unfavorable culture conditions, such as mechanical shock or other stresses. Since traps could be accused of producing similar artifacts with respect to cyst morphology and abundance, a few of the methodological aspects of this study deserve further explanation: (i) the traps were placed in a very calm location and sediment material collected in the same way as occurs with any other artificial or natural object lying in a marina. In fact, pellicle and thecate cysts were also detected in seaweed from the same station; (ii) pellicle and thecate cysts were observed in both transparent and opaque collectors, which suggests that they were not an artifactual response to the sudden absence of light; (iii) the same cysts were observed in samples without or after refrigeration, and temperature was not found to affect the viability of the cysts. Although possible effects of the traps on the sedimented *A. minutum* population cannot entirely be ruled out, the viability of the cysts and the fact that these forms were detected in the macroalgae suggest their ecological significance in the bloom dynamics of this species.

Sexual resting cysts of *A. minutum* have a mandatory dormancy period of at least 1.5 months (Figueroa et al., 2007), significantly higher than the dormancy period of the herein described pellicle and thecate cysts. Therefore, these latter cysts can be considered as short-term cysts, compared to the long-term dormancy of resting cysts. The data presented here provide strong evidence of the capacity of a small part of the *A. minutum* population blooming in Baiona Bay to encyst and then germinate during the same season, a potentially very useful short-term strategy. Compared to the fluxes of resting cysts, fluxes of the pellicle and thecate cysts were of the same order of magnitude. Thus, at least regarding cyst concentration, short-term cysts may play a significant role in the bloom strategy of *A. minutum*, as previously suggested for resting cysts (Garcés et al., 2004; Bravo et al., 2010). However, more in-depth studies are necessary to elucidate the true significance of pellicle and thecate cysts in *A. minutum* populations.

5. Conclusions

The focus of the present article was to underline the need for clarification in the terminology currently used to describe thin-walled non-dormant cysts, since there has been much confusion associated with the terms "temporary," "pellicle", and "ecdysal" when used in reference to the various types of cysts. To underline this point, data of *A. minutum* cysts from a bloom-recurrent area were presented, describing the morphologies and germination times of three different types of *A. minutum* cysts. The pellicle and thecate cysts herein reported can be considered as short-term cysts, compared to the 1.5-month dormancy period of double-walled sexual resting cysts inhabiting the same area. However, some of these thin-walled cysts have a dormancy period longer than that of resting cysts of other dinoflagellate species reported in the literature, supporting our view that the term "temporary" as a descriptor used in relation to cysts is not suitable. Moreover, the factors determining long- or short-term encystment are still unknown for many dinoflagellate species. We argue that the use of the word "temporary" to define thin-walled cysts must be definitively rejected. In addition, we recommend abandoning "ecdysal" to define thin-walled cysts because it cannot be exclusively applied to these types of cysts since thick-walled zygotic cysts (resting) also can be formed through ecdysis. Finally, we recommend "pellicle cyst" for a cyst stage in which the cyst has a single-layered wall and either no dormancy period or one that is significantly shorter than that described for resting cysts of the same species.

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